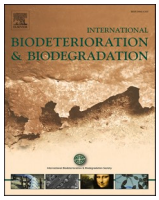
International Biodeterioration & Biodegradation 184 (2023) 105658

Contents lists available at ScienceDirect

International Biodeterioration & Biodegradation

journal homepage: www.elsevier.com/locate/ibiod



Diversity and distribution of microbial communities on the surface of External Thermal Insulation Composite Systems (ETICS) facades in residential buildings

Cristina A. Viegas [a,b,*], Giovanni Borsoi [c,d], Leonilde M. Moreira [a,b], João L. Parracha [c,d], Lina Nunes [d,e], Sofia Malanho [d], Rosário Veiga [d], Inês Flores-Colen [c]

[a] Department of Bioengineering and iBB-Institute for Bioengineering and Biosciences, Instituto Superior Técnico – Higher Technical Institute, University of Lisbon, Av. Rovisco Pais, 1049-001, Lisboa, Portugal
[b] Associate Laboratory i4HB—Institute for Health and Bioeconomy at Instituto Superior Técnico - Higher Technical Institute, University of Lisbon, Av. Rovisco Pais, 1049-001, Lisboa, Portugal
[c] CERIS, DECivil, Instituto Superior Técnico - Higher Technical Institute, University of Lisbon, Av. Rovisco Pais, 1049-001, Lisboa, Portugal
[d] LNEC, National Laboratory for Civil Engineering, Av. do Brasil, 1700-066, Lisbon, Portugal
[e] cE3c, Center for Ecology, Evolution and Environmental Changes & CHANGE, Global Change and Sustainability Institute, Azorean Biodiversity Group, University of Azores, 9700-042, Angra do Heroísmo, Azores, Portugal

ARTICLE INFO

Keywords:
Building facades
ETICS
Color/aesthetic anomalies
Bio-colonized stains
Microbiota communities
Amplicon high throughput sequencing

ABSTRACT

External Thermal Insulation Composite Systems (ETICS) are frequently used to enhance the energy efficiency of the built environment. However, stains of presumable biological nature are often detected shortly after application, causing cladding defacement and altering the building aesthetics. To address which microbiota could contribute to these biodeterioration related color/aesthetic anomalies, samples collected from stains detected on the surface of building facades with ETICS in three residential sites in Lisbon, Portugal, were analyzed through microbiological culture-dependent technique and culture-independent amplicon DNA high throughput sequencing taxonomic profiling. The obtained data provided a comprehensive description of microbial communities assigned to diverse taxa of the major microbial groups of heterotrophic bacteria, fungi, cyanobacteria, and microalgae (through DNA plastid detection) in the sampled stains. Based on that, we propose that new microorganisms could be added to the list of bio-susceptibility testing organisms in ETICS. Furthermore, microbiota diversity depended more on facade location and cardinal orientation than on ETICS material composition. Overall, this study reveals the unique microbial communities of color/aesthetic biodeterioration stains in ETICS facades, unlike those of other surfaces, and the associated environmental dynamics.

1. Introduction

External Thermal Insulation Composite Systems (ETICS) contribute to the energy efficiency of the built environment by improving the thermal insulation of the buildings' envelope, minimizing thermal bridges and consequently the risk of interior surface condensation (EAE-European Association for ETICS, 2011; Parracha et al., 2021a). These multilayer systems are composed of three different layers (the thermal insulation, the reinforced base coat, and the finishing coat) with the aim of improving the performance of the whole system (EAE-European Association for ETICS, 2011). Expanded polystyrene (EPS), mineral wool (MW) and expanded cork agglomerate (ICB) have been widely applied in ETICS as a thermal insulation layer, being EPS and MW the most common in Europe (EAE-European Association for ETICS, 2011; EAE-European Association for ETICS, 2021). The base coat consists of a mortar with several possible formulations (e.g., hydraulic binder, fillers, and organic or mineral additives) (EAE-European Association for ETICS, 2011). The finishing coat (acrylic or silicate-based) can provide not only a decorative finish to the ETICS but also weather protection (EAE-European Association for ETICS, 2011).

* Corresponding author. Departamento de Bioengenharia, Torre Sul – Piso 6, Instituto Superior Técnico, Av. Rovisco Pais, 1049-001, Lisboa, Portugal.
E-mail addresses: cristina.viegas@tecnico.ulisboa.pt (C.A. Viegas), giovanni.borsoi@gmail.com (G. Borsoi), lmoreira@tecnico.ulisboa.pt (L.M. Moreira), jparracha@lnec.pt (J.L. Parracha), linanunes@lnec.pt (L. Nunes), smalanho@lnec.pt (S. Malanho), rveiga@lnec.pt (R. Veiga), ines.flores.colen@tecnico.ulisboa.pt (I. Flores-Colen).

https://doi.org/10.1016/j.ibiod.2023.105658
Received 23 December 2022; Received in revised form 13 July 2023; Accepted 20 July 2023
Available online 25 July 2023

ETICS are generally exposed to weathering and anthropogenic factors (e.g., pollution, vandalism) which can lead to physical-mechanical (e.g., microcracks, material detachment, and material loss) and aesthetic (e.g., stains) anomalies (Amaro et al., 2013; Barreira and de Freitas, 2013). The spread of surface stains is generally driven by water, and associated with weathering and the aging of the facades (Barreira and de Freitas, 2013). Additionally, the ETICS materials properties (e.g., high-water absorption, slow drying kinetics, layer thickness), the building location, the orientation of the facade, and further anthropogenic aspects (e.g., inappropriate application, low compatibility between materials, lack of maintenance), can also influence the formation of stains (Johansson, 2011; Amaro et al., 2013; Barreira and de Freitas, 2013; Ximenes et al., 2015). Stains associated with presumable microbiological growth have been frequently detected on ETICS facades surface (Künzel et al., 2006; Johansson, 2011; Antošová, 2013; Amaro et al., 2014).

Microbial colonization of building materials is a complex phenomenon dependent on biotic (e.g., organic nutrients, activities of microorganisms, among others) and abiotic (e.g., air temperature, air relative humidity, pH, pollution, etc.) factors (Gaylarde et al., 2011; Campana et al., 2020). Material bio-receptivity is also influenced by several physical-chemical characteristics of the material surface (e.g., open porosity, roughness, chemical composition, hygroscopicity, and pH) (Barberousse et al., 2007; D'Orazio et al., 2014; Ferrari et al., 2015). In general, biofilms on the surface of building materials can contain phototrophic microorganisms (e.g., microalgae and cyanobacteria), heterotrophic bacteria, fungi, and lichens (Gaylarde and Gaylarde, 2005; reviewed in Ferrari et al., 2015). Microalgae and cyanobacteria, which use sunlight and atmospheric carbon dioxide to grow, are often identified as bio-colonization pioneers especially on external moisty and sunny surfaces, often in a mutualistic association with fungi in lichens (Gaylarde and Gaylarde, 2005; Ferrari et al., 2015; Wu et al., 2022). They can induce chromatic alterations and physical and chemical deterioration and contribute to promoting the growth of heterotrophic microorganisms (Gaylarde and Gaylarde, 2005; Ferrari et al., 2015; Komar et al., 2022; Wu et al., 2022). Many heterotrophic bacteria can metabolize organic compounds, producing metabolic molecules such as exopolysaccharides and organic acids, which can contribute to accelerating biofilm formation and substrate biodegradation (Ferrari et al., 2015). Filamentous fungi (molds) can form colored powdery stains, causing color/aesthetic alterations to building surfaces (Ferrari et al., 2015; Campana et al., 2020). In addition, fungal excretion of hydrolytic enzymes and organic acids (contributing to weakening building materials through biodegradation) combined with in-depth hyphae penetration and growth (causing mechanical micro-tensions) can induce pH alterations, discoloration, cracking, and biodegradation (Ferrari et al., 2015; Campana et al., 2020; Wu et al., 2022). The inclusion of biocides (algaecides and fungicides) in the ETICS finishing coat is currently the most adopted solution to prevent biological growth, biodeterioration and biodegradation (Reiß et al., 2021), being regulated by the EU regulation 528/2012 (EU, 2014). However, the efficacy of biocides on these coatings may decrease with time and depend on the diversity and number of the microbial colonizers, which can show variable levels of biocide tolerance and resistance (Hofbauer et al., 2011). Therefore, to be able to develop effective targeted strategies for biological growth control viewing improved ETICS maintenance and long-term durability, it is important to have a comprehensive knowledge of the microbial communities associated with the surface of the ETICS facades, which, to our knowledge, is lacking.

The detection of stains of presumable biological origin in ETICS is normally based on visual inspection of the facade (Amaro et al., 2013, 2014; Antošová, 2013). Even though visual inspection is important because it meets the users' aesthetic sensibility and expectations (Shirakawa et al., 2011), it provides no information about actual microbiota able to participate as biodeterioration agents in building materials. On the other hand, the identification of microorganisms on building materials has frequently involved microscopic and phenotypic culture-dependent techniques (Gaylarde and Gaylarde, 2005; Shirakawa et al., 2011; Ferrari et al., 2015). Culture-independent microbiological methods such as the marker gene high-throughput sequencing analyses used in the present study, allow a comprehensive identification of microbiota communities, overcoming the limitations of culture-dependent techniques (Walters et al., 2015; Karlsson et al., 2020; Savković et al., 2021). For prokaryotes, sequences of specific V3–V4 hypervariable regions of the 16S ribosomal RNA (rRNA) gene are routinely used (Walters et al., 2015; Rosado et al., 2014, 2020; Karlsson et al., 2020). For fungi, the nuclear rRNA internal transcribed spacer (ITS) region has been recognized as a suitable marker in the characterization of many fungal communities (Walters et al., 2015; Karlsson et al., 2020; Rosado et al., 2020). These methodologies have shed light on the diversity and potential for biodeterioration of microbial colonizers in, for example, building materials (Coombs et al., 2017), mural paintings (Rosado et al., 2014), artistic tiles (Coutinho et al., 2013), and (sand) stone cultural heritage buildings (Rosado et al., 2020; Wang et al., 2022; Wu et al., 2022).

Identification of microorganisms involved in the bio-colonization of ETICS in building facades is important to help predict roles microbiota may have in deterioration outcomes associated with disfigurement (e.g., color/aesthetic alterations) and/or weakness, mechanical stress, and alteration of the materials (e.g., loss of cohesion and microcracks) due to biodeterioration and biodegradation phenomena (Gaylarde and Gaylarde, 2005; Coutinho et al., 2013; Rosado et al., 2020; Wu et al., 2022). A deeper knowledge of the diversity of the microbiota thriving on these cladding systems is needed to improve bio-susceptibility diagnosis procedures and microbial biodeterioration mitigation strategies, in ETICS. Previously, Amaro et al. (2014) pointed out two major types of color/aesthetic anomalies as the most prevalent on the surface of ETICS facades during their service life (3–22 years after application), with basis on visual inspection of building facades (Amaro et al., 2014). One of the anomalies was classified as "biological growth" and generally attributed to "lichens, fungi, algae, plants" by visual observation only (Amaro et al., 2013, 2014). The other type of anomaly, classified as "runoff marks", appeared mostly below sills and parapets and was attributed to deficient application, raining events, atmospheric pollution, and dirt (dust) build-up (Amaro et al., 2013, 2014). The present study aims at identifying the taxonomic diversity and distribution patterns of the microbial communities in these types of color/aesthetic biodeterioration stains present on the surface of ETICS facades taking into consideration in-service environmental exposure, facade location/cardinal orientation and ETICS material composition.

2. Materials and methods

2.1. Study site description and meteorological data

The present study was carried out in facades of residential buildings with ETICS aged in-service from 5 to 21 years and located in three different sites in Lisbon, Portugal, hereafter designated as sites B, M, and Z, which are presented in Fig. 1 and further described below. In addition, the composition of the ETICS used in the building facades is fully described in Table 1. These ETICS have been commonly used in Europe (EAE-European Association for ETICS, 2011; EAE-European Association for ETICS, 2021; Parracha et al., 2023) and placed in the market in conformity with the Guidelines for the European Technical Approval of ETICS (ETAG, 2000; EAD, 2020). It is worth reporting that the acrylic-based paints (finishing coat) (Table 1) have biocide additives in their composition; the CAS registered number of the biocides and their initial concentrations (i.e., at the time of application in the buildings construction or retrofitting) are also reported in Table 1.

Site B (38°44′03″N, 9°12′13″W, Altitude – 110 m) is close to Monsanto Forest Park (<1 km) and to two highly congested urban freeways (Fig. 1). It consists of fourteen six-floor buildings distributed in two

Fig. 1. Location of the three sites studied in Lisbon (sites B, M and Z), Portugal, and overview of the facades where stain samples were collected in each site or area within site B. The geographical coordinates of the sites are as follows: B (38°44′03″N, 9°12′13″W, Altitude – 110 m); M (38°46′40″N, 9°05′50″W, Altitude – 16 m); and Z (38°43′34″N, 9°07′26″W, Altitude – 51 m) (credits: Google Earth).

Table 1
Composition of the ETICS and their components in accordance with the information available in building design and technical data sheets.

Sampling site	Thermal insulation (TI)	Rendering system (RS)	
		Base coat (BC)	Finishing coat (FC)
B (area 1)	Insulation cork board (ICB)	Natural hydraulic lime, mixed binders and mineral aggregates	Silicate-based, organic additives and pigments
B (area 2)	ICB	Cement and mineral aggregates	Acrylic-based, mineral aggregates, additives and pigments[a]
M	Expanded polystyrene (EPS)	Cement and mineral aggregates	Acrylic-based, mineral aggregates, additives and pigments[a]
Z	EPS	Cement and mineral aggregates	Acrylic-based, mineral aggregates and additives[b]

[a] Including terbutryn, CAS 886-50-0 (≥0.0025% to <0.025%) and isothiazole products, CAS 55965-84-9, CAS 26530-20-1, CAS 64359-81-5 (≥0.00025% to <0.0015% each).
[b] Including iodopropynyl butylcarbamate, CAS 55406-53-6 (0.05%) and isoproturon, CAS 34123-59-6 (1%).

areas, namely area 1 (Fig. 1), comprising 48 facades, built in 1990 and retrofitted in 2014 with ETICS (Table 1); and area 2 (Fig. 1), with 8 facades, built in 1998 and retrofitted in 2015 with ETICS (Table 1).

Site M (38°46′40″N, 9°05′50″W, Altitude – 16 m) is close to the river Tagus (<1 km; Fig. 1) and comprises four three-floor buildings built-in 1998 with ETICS (Table 1).

Site Z (38°43′34″N, 9°07′26″W, Altitude – 51 m), located nearby the river Tagus and close to an industrial area of the Port of Lisbon (Fig. 1), consists of two buildings with 8 floors. The thermal retrofitting of the buildings, carried out in 2002, also involved the use of ETICS (Table 1).

According to the information provided by IPMA (Portuguese Institute for Sea and Atmosphere), the meteorological data in the area of Lisbon during the two-year period of sampling (2019–2021) can be summarized as follows: the average temperature was 16.8 °C (ranging between 21.5 °C and 13.7 °C), with temperature up to 40 °C in the months of August and July; the relative humidity was 72% on average (ranging between 48% and 90%), thus rather high and favoring condensation phenomena, mostly during night hours; the average rainfall was 571 mm (mostly during Autumn); the average daily solar radiation was 16410 kJ/m^2; and, the mean wind speed was 17.5 km/h, mostly from North/West.

2.2. In-situ visual inspection

Physical-mechanical surface anomalies and stains possibly associated with biological colonization were first identified by visual analysis of the external surface of the building facades, in accordance with ASTM D3274-95 (2002) and based on the implementation of an inspection sheet previously adopted (Amaro et al., 2013). Some facades having presumable bio-colonization stains with specific visual appearance features (e.g., darkish runoff marks in sills and parapets and other colored stains covering external walls; examples in Fig. 2) were chosen to be sampled for microbiological analysis aiming at identifying bacterial and fungal communities. Detailed information on the sampling location, the description of the stains sampled, and their designations, are presented in Table 2.

2.3. Stain sample collection

One to three samples (biological replicates) were collected from the stains of possible biological origin (number of replicates from each stain and main stain characteristics in Table 2) through two different methods using minimal invasiveness, namely by scraping the surface with a sterile spatula and material collection in sterile polyethylene containers (herein called –R samples; example in Fig. 2b-insert); or, by swabbing an approximate 10 cm^2 area with sterile cotton swabs previously soaked in a sterile isotonic solution of Maximum Recovery Peptone Saline Diluent (Sigma Aldrich) supplemented with 0.1% Tween 20 (Digel et al., 2018) (herein called –S samples; examples in Fig. 2g, h, and i-insert). For isolation of culturable microorganisms, the samples were immediately processed or placed at 4 °C for around 16 h before being processed. For microbiological amplicon DNA high throughput sequencing, samples were kept at −20 °C until total DNA extraction.

2.4. Culture-dependent microbiological analysis

With the objective of assessing the abundance of culturable microbiota in the sampled stains, the detection and enumeration of culturable fungi and heterotrophic bacteria was carried out. Each sample (e.g., 0.1

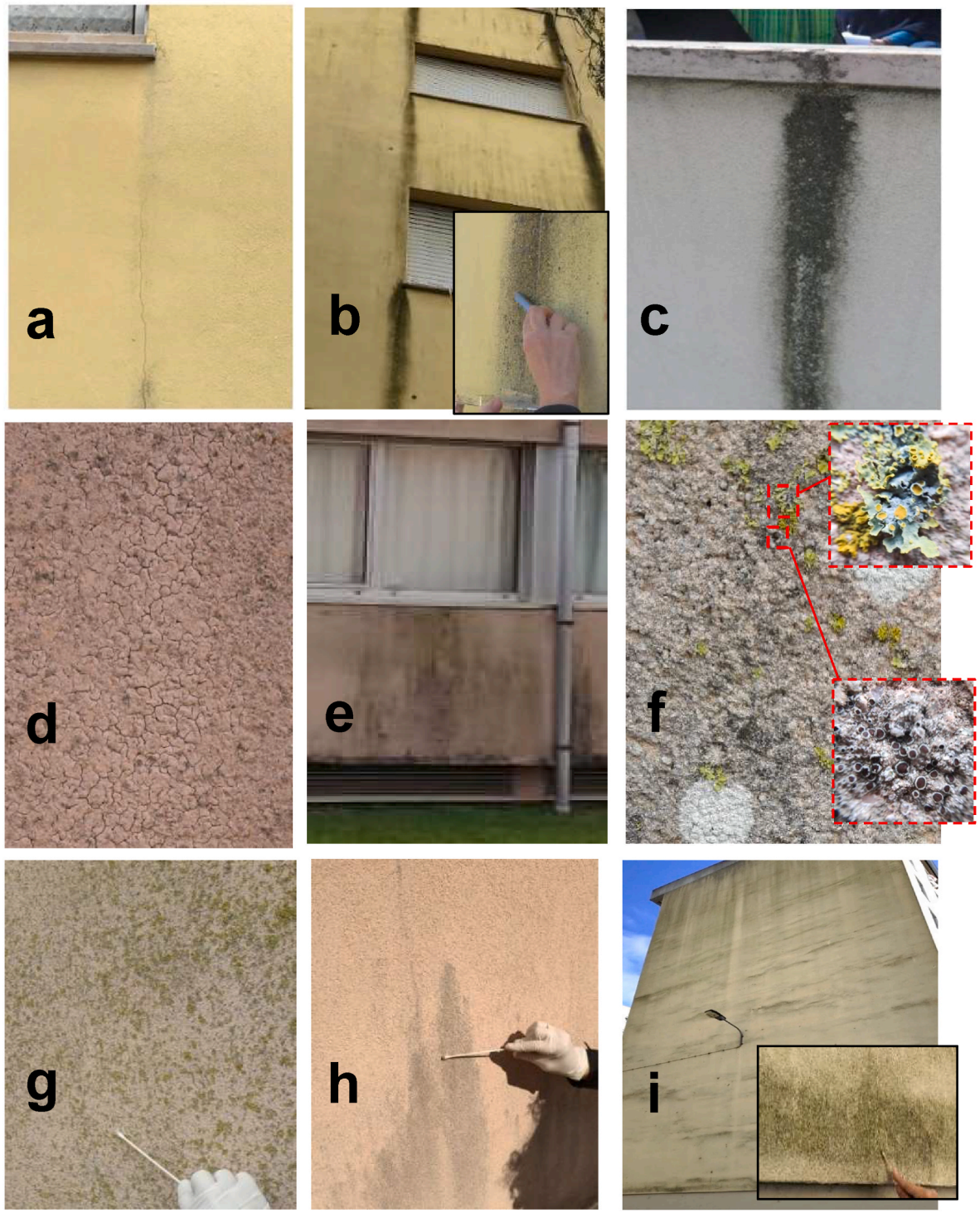


Fig. 2. Examples of anomalies and colored stains found on the surface of External Thermal Insulation Composite Systems (ETICS) facades in the sites B (a, b, c), M (d, e, f, g, h) and Z (i) and of the stains selected to be sampled. Site B: a) Vertical crack; b) brownish-blackish stains (BBS) corresponding to runoff marks bellow sills/parapets, on facades of the area 1, and detail with sample collection by scrapping; c) BBS corresponding to a runoff mark below a roof parapet in the area 2. Site M: d) microcracks; e) BBS corresponding to runoff marks bellow sills/parapets, on a North-oriented facade; f) details of lichen and possible fungal colonization obtained with magnifying objective lens for smartphone; g) greenish-yellowish stains (GYS) on a North-oriented facade and sample collection by swabbing; h) greyish stain (gS) on the surface of a South-oriented facade and sample collection by swabbing. Site Z: i) Greenish-greyish stains (GgS) and detail with sample collection by swabbing on the surface of a North-oriented facade.

g of the –R samples, or one swab cotton head of the –S samples; Table 2) was suspended in sterile Maximum Recovery Peptone Saline (1 or 0.5 mL for –R or –S samples, respectively), followed by mechanical shaking for 5 min in a vortex agitator at maximum speed. The obtained suspensions and their serial dilutions (10^{-1} and 10^{-2}, in saline solution) were spread-plated (0.1 mL; in duplicate) onto the surface of Luria-Bertani broth (LB) medium with 1.5% agar for the isolation of heterotrophic microorganisms, and Potato Dextrose Agar (PDA) supplemented

Table 2
Sampling location, major visual characteristics of the sampled stains and sample designations.

Sampling site	Facade orientation[a]	Stain characteristics	Sample designation[b] and number of samples collected from each stain (n)[c]
B	Area 1, NE	Brownish-blackish stain (BBS)	B-BBS1-R (n = 3)
	Area 1, NW		B-BBS2-R (n = 1)
	Area 2, NE		B-BBS5-R (n = 1)
M	N	Greenish-yellowish stain (GYS)	M-GYS-R (n = 2)
			M-GYS-S (n = 2)
		Brownish-blackish stain (BBS)	M-BBS-R (n = 2)
			M-BBS-S (n = 3)
	S	Greyish stain (gS)	M-gS-R (n = 2)
			M-gS-S (n = 3)
Z	N	Greenish-greyish stain (GgS)	Z-GgS-R (n = 2)
			Z-GgS-S (n = 3)
		Greenish-brownish stain (GBS)	Z-GBS-R (n = 2)
			Z-GBS-S (n = 3)

[a] NE = Northeast; NW=Northwest; N=North; S=South.
[b] According to stain characteristics and sample collection by scraping (-R) or by swabbing (-S).
[c] n = number of samples (biological replicates) collected from each stain.

with 10 mg/L of chlortetracycline (PDA + Ct medium) for selective isolation of fungi. The LB medium is a nutrient-rich and neutral pH microbial growth medium able to support the rapid growth of diverse heterotrophic bacteria and fungi. The PDA + Ct medium is recommended for the isolation of fungi (molds and yeasts) (Mislivec and Bruce, 1976). The inoculated media were incubated at room temperature (20 ± 5 °C) with indirect sunlight radiation (simulating natural night/day periods) for 5 days, after which the colonies formed were counted and photographed. Fungal colonies isolated on PDA + Ct medium were initially visually distinguished based on their macroscopic features (e.g., color, form, size, and texture), and the number of each colony-type was counted. Culturable population abundance was expressed as the number of colony-forming units (CFU) per sample.

2.5. Phylogenetic identification of culturable fungi

The most prevalent (i.e., found at ≥ 10 CFU/sample) and morphologically distinct filamentous fungi colonies detected on PDA + Ct medium (section 2.4.), were subcultured on PDA medium to obtain pure cultures. Their taxonomic phylogenetic identification was performed by the analysis of the partial sequences of the rRNA ITS1/ITS2 region. For this purpose, one to three colonies were picked up from the pure cultures in PDA and frozen at −20 °C until genomic DNA extraction. Total DNA was extracted from the thawed fungal biomass by using the Nucleospin Soil DNA extraction kit (Mackerey-Nagel, Germany) with SL1 lysis buffer (700 μL), lysis enhancer SX (150 μL), homogenization in a vertical TurboMix Disruptor Génie (Scientific Industries, Inc, US) for 8 min, and further steps according to the kit manufacturer's instructions. DNA concentration and quality were examined in a Nanodrop spectrophotometer. The ITS region of each fungus was amplified with primer pair ITS1f and ITS2r (Walters et al., 2015) and Platinum™ II Hot-Start PCR Master Mix (Thermo Fisher Scientific, Lithuania), following the manufacturer's instructions. Thermocycling conditions consisted of 94 °C for 2 min (initial denaturation), followed by 35 cycles of denaturation at 94 °C (30 s), annealing at 60 °C (30 s), and extension at 68 °C (30 s), and a final extension at 68 °C for 10 min. The presence of the amplicon (approximately 400 bp) was checked by agarose gel (0.8% w/v) electrophoresis. Each PCR product was purified using the DNA Clean and Concentrator-25™ kit (Zymo Research Europe, Germany), checked for concentration and purity in a NanoDrop spectrophotometer, and sequenced in the GATC Biotech AG laboratory (Germany) using the Sanger method. For the taxonomic classification of each fungus, the obtained sequences were manually corrected to ensure that the base calling algorithm has assigned proper base ascription, and further subjected to homology search using BLASTN alignment in the NCBI (National Center for Biotechnology Information) platform and in the User-friendly Nordic ITS Ectomycorrhiza (UNITE) database (Nilsson et al., 2019). Sequences producing significant alignments with ≥98% identity and not more than 1 nucleotide difference in length (i.e., insertion or deletion of one nucleotide) between the query and the reference sequences, in at least one of these two platforms, were used to infer fungal isolate identification at the genus level.

2.6. Total DNA extraction from the stain samples, processing, and amplicon high throughput sequencing

To obtain the taxonomic profiles in the microbial communities present in the samples, a culture-independent methodology based on amplicon DNA high throughput sequencing of the stain's microbiota was carried out. Analysis of the sequences of the V4 variable regions of 16S rRNA genes gave information regarding bacterial community profiles and presence of plastids (chloroplast) from microalgae, whereas fungal community profiles were obtained from the analysis of the sequences of amplicons corresponding to ITS1/ITS2 rRNA regions. Total DNA was extracted from each sample (0.1 g or one swab head of the –R or –S samples, respectively) by using the NucleoSpin Soil DNA Extraction kit with SL2 lysis buffer plus 15 μL or 100 μL lysis enhancer SX for –R or –S samples, respectively, and further procedure as described in section 2.5. Total DNA integrity was checked by agarose gel (0.8% w/v) electrophoresis. Upon examination of concentration and quality, DNA solutions obtained from replicas of the same stain (Table 2) were pooled. For bacterial community and plastid analysis, the DNA samples were sent to the Genomic Unit of Instituto Gulbenkian de Ciência (IGC; Oeiras, Portugal) for amplicon high throughput sequencing; 16S rRNA gene was amplified targeting the hyper-variable V4 region using the forward primer 515F (5′-GTGYCAGCMGCCGCGGTAA-3′) (Parada et al., 2016) and the reverse primer 806R (5′-GGACTACNVGGGTWTCTAAT-3′) (Apprill et al., 2015). PCR reaction setup included the Platinum Hot Start enzyme and PCR master mix (2x) (ThermoFisher). The cycling conditions consisted of an initial denaturation at 94 °C for 3 min, followed by 35 cycles of denaturation at 94 °C (1 min), annealing at 50 °C (1 min), extension at 72 °C (105 s), and a final extension at 72 °C for 10 min. The library was mixed with PhiX control and sequenced by using the Illumina MiSeq platform in a paired-end run (2x250 cycles V3 kit). For fungal community analysis, amplicons targeting the fungal ITS regions were prepared from the DNA samples (2–6 μL template DNA/-reaction) by using PCR hybrid primers flanked by Illumina universal primer sequencing for barcode insertion, for ITS1f (5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAGCTTGGTCATTTAGA-GGAAGTAA-3′) and ITS2r (5′-GTCTCGTGGGCTCGGAGATGTGTATAA GAGACAGGCTGCGTTCTTCATCGATGC-3′) (Karlsson et al., 2020), using the Platinum™ II Hot-Start PCR Master Mix and the cycling conditions described in section 2.5., following manufacturer's instructions. Upon amplicon confirmation by agarose gel electrophoresis and purification (as described in section 2.5), the amplicons were sent to the IGC's Genomic Unit for sequencing. The amplicons were subjected to an index PCR to attach the dual indices and Illumina sequencing adapters using the Nextera XT Index Kit according to manufacturer instructions. After combining the amplicon library with PhiX control, samples were sequenced using the Illumina MiSeq platform in a paired-end run (2x300 cycles V3 kit).

2.7. Amplicon DNA high throughput sequence analysis

With the aim of identifying the microbial phylogenetic taxonomic groups in each sampled stain, pipeline QIIME 2 2017.4 was used for sequences clustering into Operational Taxonomic Units (OTUs) based on a percentage identity with known taxa within specific databases (Bolyen

et al., 2019). Raw sequence data were demultiplexed and quality filtered using the q2-demux plugin (feature count for each sample in Supplementary materials Table S1). The demultiplexed sequences were denoised with DADA2 (Callahan et al., 2016) embedded in QIIME2, and sequence variants (SVs) were detected. The taxonomic identification for SVs was subsequently performed using the naive-Bayesian classifier (Bokulich et al., 2018) trained at 99% identity in the Greengenes 13_8 database in the case of the bacterial 16S rRNA amplicons (McDonald et al., 2012) or at 97% identity at the UNITE (version 9.0, dynamic) database for the fungal ITS sequences (Abarenkov et al., 2021).

2.8. Nucleotide sequences

Fungal colony sequences were deposited in the NCBI GenBank (please see accession numbers in Table S2 in Supplementary materials). The raw nucleotide sequences of the microbiomes were deposited in the Sequence Read Archive under the BioProject accession ID PRJNA912626.

3. Results

3.1. Visual inspection

Building facades from the three residential sites in Lisbon (sites B, M, and Z) were inspected for visible anomalies (examples in Fig. 2). In site B, the facades generally presented finishing coat detachment mainly in the edges and in the window interfaces, visible cracks in the proximity of the windowsills (Fig. 2a), and abundant stains presumably associated with bio-colonization (Fig. 2b and c). Concretely, in area 1 of site B (described in section 2.1; Fig. 1), brownish-blackish stains (BBS) were observed on the surface of the Northwest (NW) and the Northeast (NE) oriented facades (Table 2), mostly corresponding to runoff marks in water runoff spots below or in the proximity of roof parapets and window-sills (Fig. 2b). On the other hand, presumable bio-colonization stains were almost absent in the South oriented facades (not shown). Conversely, the buildings in area 2 (section 2.1; Fig. 1) presented noticeably lower incidence of this type of stain, and almost solely below roof parapets in the NE-oriented facades (Fig. 2c; Table 2). In general, the ETICS facades in area 2 of site B showed a considerably better conservation condition than in area 1 (Fig. 1 and data not shown).

Blistering and microcracking of the finishing coat were observed in some areas of site M (Fig. 2d), mostly in the proximity of the windowsills, the corner brackets, and the joints among the insulation boards. Three different types of presumable bio-colonization stains were detected in several spots of the site M buildings (Fig. 2e–h). For example, brownish-blackish stains (BBS) corresponding to runoff marks below sills/parapets (Fig. 2e) and greenish-yellowish stains (GYS) covering wall surface (Fig. 2g) were found mostly on the North (N)-oriented facades (Table 2). In general, the darkish BBS predominated in the lower parts of the facades facing the yard, in the proximity of high humidity conditions, possibly associated with water surface runoff, capillary rise, weathering, and/or water condensation and accumulation (Fig. 2e), whereas the GYS (Fig. 2g) were mostly covering upper and apparently less humid areas of the facades. Comparatively, these two types of stains were found absent from the South (S) oriented facades, where however a less colored greyish stain (gS) of possible biological growth was observed (Fig. 2h; Table 2).

On the ETICS facades of site Z, anomalies were detected such as cracks, mostly in the windowsills, discoloration (data not shown), as well as more uniformly distributed stains of possible biological growth. The latter stains were predominantly colored greenish-greyish (GgS) or greenish-brownish (GBS) (Table 2) and covered the surface of the N-oriented facades (Fig. 2i).

Inspection thus indicated the presence of several visible signs of color/aesthetic biodeterioration anomalies presumably associated with microbial colonization in the ETICS facades of the three sites (Fig. 2). Therefore, one representative facade of each site was selected for the collection of stain samples (details in Table 2) for microbiological analysis.

3.2. Detection and phylogenetic identification of culturable microorganisms

A survey of culturable heterotrophic microorganisms in the samples collected from the surface of the ETICS facades (Table 2) revealed the presence of considerable numbers of diverse microbial CFU in the stains of the three sites (Fig. 3, and Fig. S1 in Supplementary material). The total number of heterotrophic CFU able to grow in LB medium was slightly higher in the stain samples of site B than in the stains of sites M or Z, notwithstanding the mode of sample collection by scrapping (samples –R) or swabbing (samples –S) for the same stain (Fig. 3a).

Regarding the culturable filamentous fungi, eight types of colonies, hereafter named A, B, C, D, E, F, G and H, with different macroscopic characteristics (in terms of color, form, size and/or texture) were detected as the most prevalent isolates within the set of stain samples analyzed (Fig. S2 in Supplementary materials). The relative CFU numbers of these major fungi found in the bio-colonized stain samples from the sites B, M, and Z, are compared in Fig. 3b. In general, the profiles of culturable fungi varied slightly between bio-colonized stain and site location (Fig. 3b). For instance, colony-type A fungi (Fig. S2) was found in the samples from the site B only, mostly in the stains B-

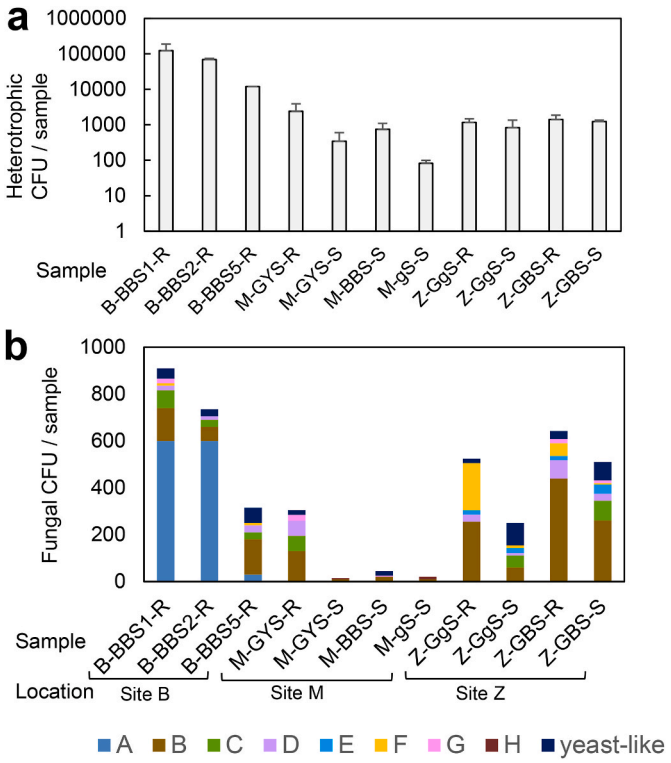


Fig. 3. Culture-dependent microbiological analysis of the stain samples collected from the External Thermal Insulation Composite Systems (ETICS) facades of the sites B, M and Z (as described in Table 2). Graphics represent colony forming units (CFU) numbers of a) heterotrophic microorganisms enumerated by spread-plating the samples onto agarized LB medium, and b) the most prevalent colony-types of fungi enumerated on PDA + Ct medium, for each of the indicated samples. In a), error bars indicate +1 standard deviation from duplicated CFU determinations for one or two replicated stain samples. In b), the letters A to H refer to the eight most prevalent distinct colony-types of filamentous fungi detected within the set of stain samples analyzed (shown in the Supplementary Fig. S2) and further subjected to phylogenetic identification at genus-level (as detailed in the Supplementary Table S2).

BBS1 and B-BBS2 of the area 1 (Fig. 3b), whereas the colony-types G and H (Fig. S2) were detected mostly in the M-GYS and M-gS samples from the site M and the Z-GBS of the site Z (Fig. 3b). On the other hand, the fungi of colony-types E and F (Fig. S2) prevailed mostly in the stains of the site Z (Fig. 3b). Notably, mold isolates of the colony-types B, C, and D (Fig. S2) were found in all the stain samples of the three sites (Fig. 3b). Several yeast-like colonies, with whitish or pinkish color, were also detected in almost all samples (Fig. 3b; Fig. S2). The total CFU numbers of fungi were on average lower in the samples from facades of site M than in the other two sites, whereas the highest values were found in the samples from area 1 of the site B (Fig. 3b). However, a possible influence of the mode of sample collection in the numbers of the detected fungal colonies should not be ruled out, because the total fungal CFU numbers were generally lower in the –S samples as compared to the –R samples for the same stain (Fig. 3b).

The phylogenetic taxonomic classification of the colonies A, B, C, D, E, F, G, and H based on the ITS region sequence analysis pointed out these filamentous fungi belonged to the genera *Didymellla* (A), *Cladosporium* (B, C and D), *Aureobasidium* (E), *Penicillium* (F), and *Epicoccum* (G and H) (details in the Supplementary Table S2).

In summary, the culture-dependent analysis evidenced the prevalence of several genera of culturable fungi as well as diverse non-identified heterotrophic bacteria in the ETICS bio-colonized stains under study. Notably, filamentous fungi of the genus *Cladosporium* were prevalent in all the stains.

3.3. Characterization of the microbial communities based on amplicon DNA high throughput sequencing

The culture-independent assessment of taxonomic diversity based on total DNA extracted from each stain sample followed by amplicon high throughput sequencing provided a more comprehensive picture of the microbiota composition. Fig. 4 shows the relative abundance at phylum-level (Fig. 4a) and genus-level (Fig. 4b) of the predominant populations identified by 16S rRNA sequencing analysis in the samples collected from the ETICS facades in the sites B, M and Z. All the sampled bio-colonized stains held bacterial populations belonging to six main phyla, with Operational Taxonomic Units (OTUs) abundance in the order: *Proteobacteria* > *Cyanobacteria* > *Bacteroidetes*, *Actinobacteria, Thermi* > *Acidobacteria* (Fig. 4a). In almost all sampled stains less than 2% of the OTUs detected were unassigned bacteria; this number was slightly higher (~5%) for the bio-colonized stain B-BBS5 (Fig. 4). A total of 32 genera were detected (about 80% of them identified at least at the family-level) in the bio-colonized stains (Fig. 4b). In general, the bacterial community compositions at genus level appeared to be relatively consistent within each site, showing significant differences between the three locations (Fig. 4b). In addition, in some cases (e.g., sites B or M), the results showed considerably distinct bacterial community profiles between stains with different visual appearance (Fig. 2; Table 2) in the same location, as for example for the stains M-BBS, M-GYS and M-gS of the site M (Fig. 4b; Supplementary Table S3). Interestingly, the brownish-blackish stains (BBS) sampled from runoff marks below window sills in site M (e.g., M-BBS) appeared to have a bacterial profile

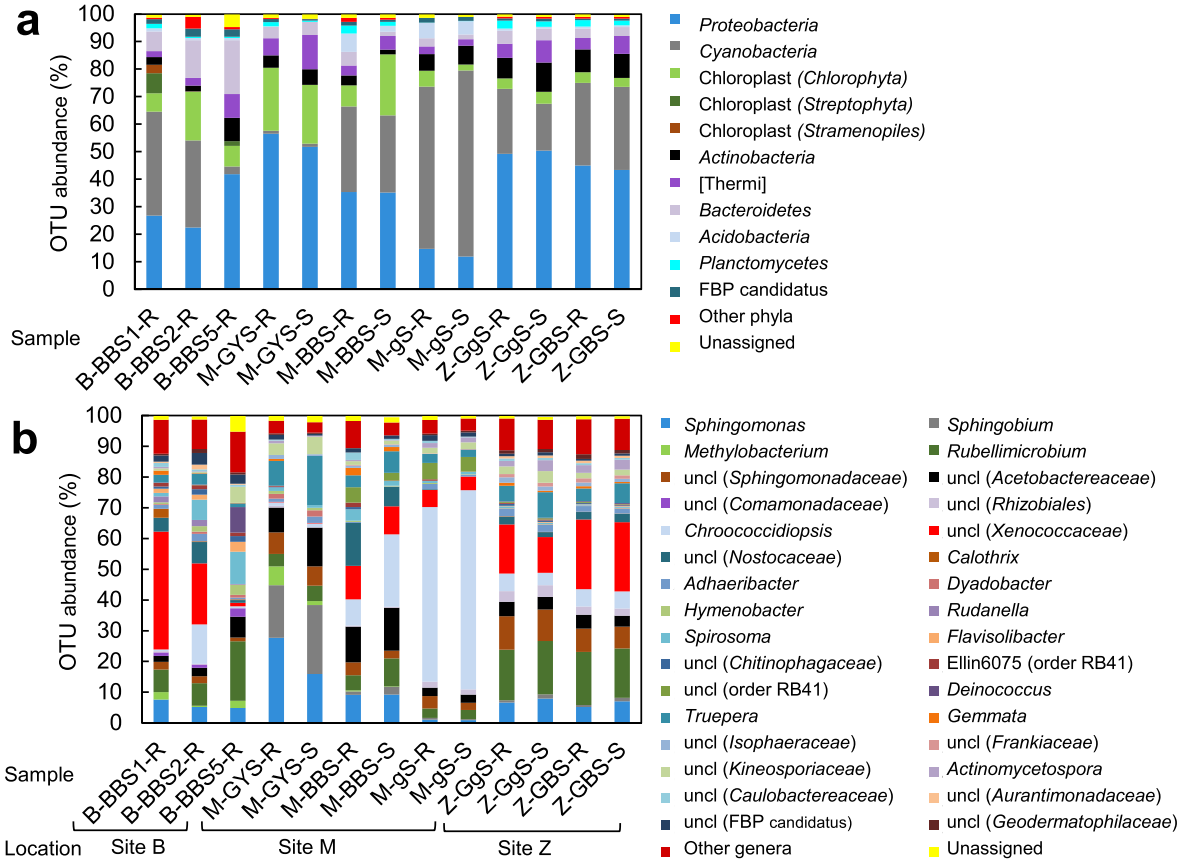


Fig. 4. Composition of microbial communities at the a) phylum-level and b) bacterial genus-level, expressed as % of Operational Taxonomic Units (OTUs) abundance, obtained by 16S rRNA gene sequence analysis. Amplicons were obtained from total DNA extracted from the stain samples collected from the External Thermal Insulation Composite System (ETICS) facades of the sites B, M, and Z (as described in Table 2). The most representative a) phyla or b) bacterial genera are indicated on the right side of the charts. For each unclassified (uncl) genera, the lowest taxonomic level identified is indicated between brackets. In a) and b), taxa with OTUs abundance below 1% in all samples are collectively indicated as "Other phyla" and "Other genera", respectively.

much closer to the stains with identical visual appearance sampled from runoff marks in site B (e.g., B-BBS1 and B-BBS2) than to the other stains (e.g., M-GYS and M-gS; Table 2) detected in the same site M (Fig. 4b). Notably, the less colored greyish stain (e.g., M-gS) from the South-oriented facade of site M (Fig. 2, Table 2) showed a unique bacterial taxonomic profile, namely with very low abundance of *Proteobacteria* genera (<13%) and high prevalence of OTUs assigned to the phylum *Cyanobacteria* (mostly of the genus *Chroococcidiopsis*) (abundance >59%) as compared to all the other bio-colonized stains where this taxon was considerably less represented (namely, <3% abundance in the BBS5 and GYS stains of the sites B and M, respectively, or 21–38% in the other stains) (Fig. 4a and b). Further details regarding the comparison of the most abundant bacterial taxa (in the hierarchy Phylum_Family_Genus) found in each bio-colonized stain are shown in Supplementary Table S3. In addition, when analyzing the mode of sample collection, no significant differences were observed between the –S or –R samples (Table 2) collected from the same stain in the sites M or Z (Fig. 4a and b; Supplementary Table S3).

The 16S rRNA sequencing analysis also indicated the presence of a considerable number of OTUs assigned to plastids (e.g., chloroplasts) of green microalgae belonging to the phylum *Chlorophyta*, which were found in all the bio-colonized stain samples (Fig. 4a). However, these OTUs were more abundant in the runoff brownish-blackish stains (BBS) of the site B (6.7–17.5% relative abundance) and in the BBS and the greenish-yellowish stains (GYS) of the site M (7.7–22.1% and ~22.9%, respectively) than in the other bio-colonized stains (<5.5%) under analysis (Fig. 4a). It is worth noting that greenish-colored lichens could be also visualized within these stains (Fig. 2). In addition, plastids of the plant and green microalgae clade *Streptophyta* and of the phylum *Stramenopiles* were also represented in the bio-colonized stains from the site B (<2.9% relative abundance), being however absent in the stain samples of the other two sites (Fig. 4a).

The relative frequency of fungal taxa at order-level found in the bio-colonized stains is shown in Fig. 5. While analyzing the mode of sample collection, one can observe that the fungal taxonomic profiles were, in general, very similar between the –S or –R samples collected from the same stain (Fig. 5). As it concerns the fungal community in the sampled stains, it was clearly dominated by taxa belonging to the phylum *Ascomycota* (Fig. 5, and Supplementary Table S4). In general, results showed taxonomic profiles relatively consistent within each site, with differences between the three locations (Fig. 5). However, in some cases (e.g., for sites B or M), the fungal community profiles were significantly different between different stains within the same location (Fig. 5). For example, regarding the runoff brownish-blackish stains (BBS) sampled in the site B, the ones collected in the facades of the area 1 (B-BBS1 and B-BBS2; Table 2) showed fungi profiles closer to each other and clearly distinct from the B-BBS5 collected from the area 2 (Fig. 5). In addition, the fungal taxonomic profiles in the runoff BBS and the greenish-yellowish stains (GYS) sampled in the North-oriented facades of the site M (samples M-BBS-R/M-BBS-S and M-GYS-R/M-GYS-S, respectively; Table 2) showed some differences between each other, namely much higher percentages of OTUs assigned to the orders *Chaetothyriales* and *Candelariales* in the BBS than in the GYS (Fig. 5). In addition, the fungal taxonomic profile in the greyish stain from the S-oriented facade of the site M (samples M-gS-R and M-gS-S; Table 2) was considerably different from the other four samples of the same site (described above); indeed, the gS samples showed lower taxonomic diversity, mostly dominated by taxa of the order *Chaetothyriales* (family *Trichomeriaceae*/genus *Lithohypha*) (Fig. 5, and Supplementary Table S4). However, it is worth noting that about 45% of the fungal populations remained undefined at the phylum and lower levels in two samples (Fig. 5), which may be associated with the prevalence of one or more not yet classified fungi or other factors not disclosed at the present time. Further details regarding the most abundant fungal taxa (in the hierarchy Phylum_Class_Order_Family_Genus) in each bio-colonized stain from sites B, M, and Z are available in Supplementary Table S4. In summary, results indicated the mold genera *Cladosporium*, *Lithohypha*, *Flavoplaca*, and *Xanthoria* to be present in all the bio-colonized stains analyzed (Table S4). Other highly represented genera were *Neodevriesia* (77% of the samples; mostly sites B and M), *Alternaria* (69%; mostly sites B and Z), *Cyphellophora* (69%; mostly B and Z), *Coniosporium* (69%; mostly sites B and M), *Knufia* (53%; mostly B and Z), *Epicoccum* (46%; M and Z), *Lecanora* (46%; site M), *Vermiconia* (53%; site M), and *Extremus* (53%; site M) (Table S4).

Overall, all the stains corresponding to darkish runoff marks and other color/aesthetic stains on ETICS facades comprised diverse taxa of fungi (Fig. 5; Table S4), heterotrophic bacteria (Fig. 4; Table S3), cyanobacteria (Fig. 4; Table S3), and microalgae (Fig. 4a). Interestingly, the bio-colonized stains in the surface of ETICS with similar composition, like it is the case of the facades in sites M and Z (e.g., comprising EPS as thermal insulation, cement/mineral aggregates as the base coat, and acrylic-based finishing coat; Table 1), showed considerably distinct bacterial (Fig. 4b) and fungal (Fig. 5) taxonomic community profiles. Therefore, the differences in the respective microbiota can barely be related to the specific composition of the ETICS. Nevertheless, some degree of influence of ETICS composition should not be ruled out, because the microbiota profiles in the bio-colonized stains of site B diverged significantly from the latter ones (sites M or Z) but also between the areas 1 and 2 facades (Figs. 4b and 5); indeed, ETICS in the site B facades comprised different finishing coat (silicate-based or acrylic-based in areas 1 or 2, respectively), base coat (natural hydraulic lime or cement/mineral aggregates in areas 1 or 2, respectively) and thermal insulation (ICB) materials (Table 1). In terms of environmental exposure, the site B is located close to the Monsanto Forest but also in the

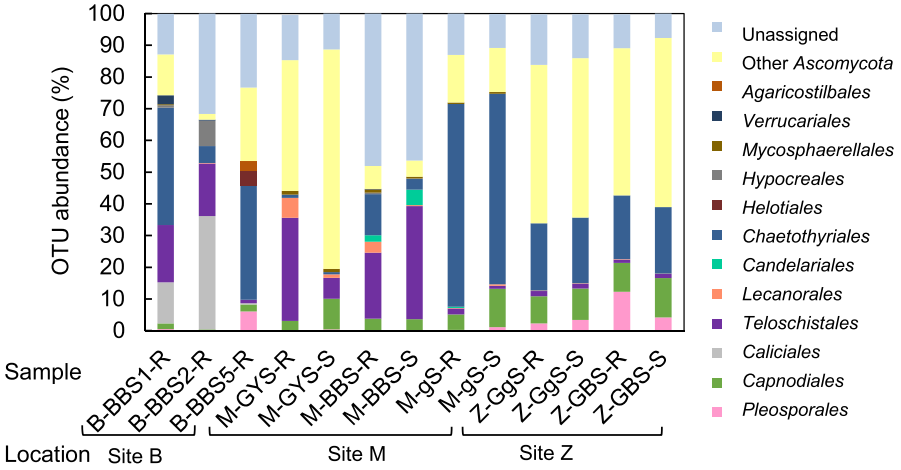


Fig. 5. Fungal community composition at the order-level, expressed as % of Operational Taxonomic Units (OTUs) abundance, obtained by the internal transcribed spacer (ITS) ribosomal RNA region sequencing analysis. The ITS amplicons were obtained from total DNA extracted from the stain samples collected from the ETICS facades of the sites B, M, and Z (as described in Table 2). The most representative fungal taxa at order-level are indicated on the right side of the chart. Unidentified taxa and taxa with abundances below 0.5% within the phylum *Ascomycota* are collectively indicated as "Other *Ascomycota*".

proximity of two heavily congested urban freeways (Fig. 1), while the sites M and Z are located closer to river Tagus and in less traffic-congested residential areas (Fig. 1). On the other hand, a possible influence of facade cardinal orientation on bacterial and fungal taxonomic diversity, is evidenced by the taxonomically richer microbiota generally found in the bio-colonized stains formed in the Northeast (NE)-/Northwest (NW)-/North (N)-oriented facades (e.g., BBS in the sites B and M, GYS in site M, and GgS/GBS in site Z) than in the greyish stain (gS) in the South (S)-oriented facades of site M (Figs. 4 and 5). Interestingly, despite the microbiota diversity and the indicated differences found in the microbial community profiles between stain samples (Figs. 4b and 5), a set of core taxa was common to all the analyzed color/aesthetic anomalies, namely: (i) *Chorophyta* green microalgae (Fig. 4a); (ii) cyanobacteria of the family *Xenococcaceae* (Fig. 4b; Table S3); (iii) alphaproteobacteria of the families *Sphingomonadaceae*, *Acetobacteraceae*, *Rhodobactereaceae, Cytophagaceae* and *Trueperaceae* (Fig. 4b; Table S3); (iv) actinobacteria of the family *Kineosporiaceae* (Fig. 4b; Table S3); and (v) molds of the genera *Cladosporium*, *Lithohypha*, *Flavoplaca*, and *Xanthoria* (Fig. 5; Table S4).

4. Discussion

4.1. ETICS stains' microbiota uniqueness and proposition of new bio-susceptibility testing microorganisms

The comprehensive knowledge on the microbiota colonizing biodeterioration-related color/aesthetic stains, achieved with the present work, is a step forward to refining microorganism selection in the implementation of bio-susceptibility testing methods in ETICS. For instance, to our knowledge, the few studies which have addressed the bio-susceptibility of ETICS materials focused on a limited number of mold species (e.g., *Aspergillus niger* and *Penicillium funicolosum*, according to standard test methods for paints and insulation materials, in Parracha et al., 2021a,b) or microbial phototrophs (e.g., the cyanobacterium *Chroococcidiopsis fissurarum*, and several microalgae species, in Barberousse et al., 2007; D'Orazio et al., 2014), as standard biodeterioration agents. Our analyses suggest this bio-susceptibility testing in ETICS would gain with the inclusion of other microorganisms belonging to diverse taxa, selected for instance within the ones found in common in all the ETICS bio-colonized stains. For example, *Cladosporium* spp. could be proposed as test-fungi, given that it was found recurrent in all the stains studied. Similarly, heterotrophic bacteria of the phyla *Actinobacteria* and *Alphaproteobacteria,* with potential to contributing to biodeterioration processes (discussed in 4.3.), could also be considered as additional test-microorganisms. Besides, our results corroborate the importance of *Chroococcidiopsis* spp. and green microalga (*Chlorophyta*) as standard phototrophic microorganisms in bio-receptivity and biodeterioration testing in ETICS (Barberousse et al., 2007; D'Orazio et al., 2014).

In the present work, an apparently unique feature of the microbiota colonizing the ETICS colored/aesthetic stains was the absence of several bacteria (e.g., families *Bacillaceae*, *Clostridiaceae*, *Rhizobiaceae*, *Xanthomonadaceae*, *Pseudomonadaceae, Micrococcaceae*) and fungal taxa (e.g., *Aspergillus*, *Acremonium*, *Ulocladium*, *Fusarium,* etc.). This finding was relatively surprising because these are ubiquitous airborne and soil/rock-inhabiting microorganisms that can be dispersed through the air in dust (Karlsson et al., 2020; Savković et al., 2021) and have been frequently found colonizing building facades worldwide (Campana et al., 2020; Rosado et al., 2020; Wang et al., 2022). We suggest this may be related with, for instance, the surface properties (hydrophobicity, rugosity, etc.) of the ETICS (D'Orazio et al., 2014; Parracha et al., 2021a) and/or local environmental and climatic factors. Nevertheless, it is worth mentioning that, in general, the main bacteria and fungi taxa identified in the color/aesthetic anomalies of the ETICS facades in the three sites were not considerably distinct from the main microbial taxa that have been reported as contributing to the biodeterioration and biodegradation of other historic and modern building exteriors worldwide, like for instance in Europe (Gaylarde and Gaylarde, 2005; Coutinho et al., 2013; Rosado et al., 2020), Asia (Wu et al., 2022; Wang et al., 2022) or Latin America (Gaylarde and Gaylarde, 2005; Shirakawa et al., 2011).

4.2. Environmental and ETICS materials related factors in bio-colonization of ETICS stains

Taxonomic profiles of the bacterial and fungal communities appeared to be more related to the stain type (visual appearance) than to specific factors associated with the ETICS material composition, as it is evidenced by, for instance, the distinct microbiota profiles of the runoff brownish-blackish stains (BBS), the greenish-yellowish stains (GYS) and the greyish stains (gS) within facades with the same ETICS components in the same location (site M). In addition, the influence of facade cardinal orientation (e.g., N-/NE-/NW-oriented *versus* S-oriented facades in the site M) may be associated with the respective environmental exposure conditions, since higher sunlight exposure and drying rate of the S-facades may limit microbial growth (Coutinho et al., 2013). In particular, the outstanding dominance of *Chroococcidiopsis* spp. within the microbiota of the S-facade can be related to the known high resistance to photooxidation, periods of desiccation, and extreme temperatures of these cyanobacteria (Gaylarde and Gaylarde, 2005; Nienow, 2009). On the other hand, higher surface condensation may occur in the N-/NE-/NW-oriented facades studied, due to high humidity conditions and the wind blowing mainly from N-NW in the region of Lisbon (Parracha et al., 2022), making them more prone to the formation of stains of biological origin. However, other local environmental factors may influence facade microbial colonization, possibly contributing to some differences in microbiota community composition between the stains in sites B, M or Z. For example, the proximity of the site B to the Monsanto Forest may influence the microbial community diversity due to dispersal of airborne particles possibly containing organic substrates and microorganisms from the surrounding soil and diverse vegetation (Coutinho et al., 2013, 2015; Savković et al., 2021). On the other hand, two heavily congested urban freeways also in the proximity of site B can be a source of pollutants (e.g., SO_x, NO_x, CO, hydrocarbons, etc.) possibly contributing to surface anomalies like darkish runoff marks (Amaro et al., 2014) and increased bio-receptivity (Shirakawa et al., 2011; Parracha et al., 2021b). Comparatively, the sites M and Z are located closer to river Tagus and within less traffic-congested residential areas.

Therefore, we suggest that ETICS bio-receptivity and bio-colonization, the taxonomic diversity of the microbial communities able to thrive on it, and thus their potential for biodeterioration, may be linked to a combination of several factors, making it rather difficult to discriminate one or two major contributing factor(s). Those factors can be related with ETICS materials, local nutrient availability, facade orientation, local microclimate conditions (e.g., wind direction, sunlight incidence, and intensity, moisture), air pollution, and/or biota living in the surroundings, as suggested by others reporting building materials bio-susceptibility (Antošová, 2013; Gaylarde et al., 2011; Parracha et al., 2021b) and microbial growth on the exteriors of buildings and cultural heritage monuments (Coutinho et al., 2013; Gaylarde and Gaylarde, 2005; Shirakawa et al., 2011; Wang et al., 2022; Wu et al., 2022).

In addition, uncontrollable variables associated with inadequate anti-microbial protection of the ETICS finishing coat (Amaro et al., 2013) due to possible leaching, biodegradation (by bacteria and fungi) and/or chemical/photochemical degradation of organic biocide additives over time (Burkhardt et al., 2011; Hofbauer et al., 2011; Reiß et al., 2021), may also have contributed to ETICS microbial bio-colonization. The biocide additives initially incorporated in the ETICS acrylic-based finishing coat at the time of application in the buildings construction or retrofitting may have disappeared, totally or partially, and/or lost their activities, because the ETICS facades service-life was approximately 5, 21 and 19 years in the sites B/area 2, M and Z, respectively, at

the time the present work was carried out. Indeed, Burkhardt et al. (2011) reported organic biocide concentration to tend to zero, becoming non-effective, after more than 5–7 years from the application of the finishing coats, due to leaching from construction materials to raining water runoff (Burkhardt et al., 2011). Possible failure of biocides during facade in-service environmental exposure, leading to bio-colonization and color/aesthetic anomalies, can have important implications for the building materials coatings industry (Gaylarde and Gaylarde, 2005) and regarding aesthetic appeal, maintenance, and long-term durability of ETICS facades.

4.3. Potential contributions to biodeterioration phenomena of prevalent ETICS facade bio-colonizers

The main microbial phototrophs found in the ETICS facade stains (e.g., cyanobacteria mostly of the genus *Chroococcidiopsis*/family *Xenococcaceae*, and *Chlorophyta* green microalgae) have been reported as biodeterioration agents frequently found in building exteriors (Gaylarde and Gaylarde, 2005; Crispim et al., 2006; Coutinho et al., 2015; Ferrari et al., 2015; Komar et al., 2022; Wu et al., 2022). In terms of biodeterioration potential, their darkish, bluish, greenish, or brownish pigmentation may contribute to the color/aesthetic anomalies (Gaylarde and Gaylarde, 2005; Shirakawa et al., 2011; Ferrari et al., 2015). In addition, since these microorganisms are primary producers of carbohydrates and many cyanobacteria species are nitrogen-fixing, they can produce substrates for microbial growth, with the potential to promote the establishment of heterotrophic bacteria and fungi (Gaylarde and Gaylarde, 2005; Shirakawa et al., 2011; Ferrari et al., 2015; Komar et al., 2022; Wu et al., 2022).

On what concerns heterotrophic bacteria as colonizers and potential biodeterioration agents in the ETICS facades, many of the alphaproteobacteria (e.g., families *Rhodobactereaceae, Acetobactereaceae,* and *Sphingomonadaceae*), actinobacteria (e.g., *Kineosporiaceae*, *Pseudonocardiaceae,* and *Frankiaceae*), bacteroidetes (e.g., *Cytophagacea*e) and Deinococcus-Thermus taxa (e.g., *Truepera*) found prevalent in the ETICS stains' microbiota, have been also detected in biofilms of other building surfaces (Gaylarde and Gaylarde 2005; Coutinho et al., 2013, 2015; Rosado et al., 2020; Wang et al., 2022). These bacteria are generally ubiquitous in nature (soil, water, and plants) and can undergo airborne dispersal by sticking to dust particles, ultimately ending up on surfaces where they can establish and grow (Gaylarde and Gaylarde, 2005; Denner et al., 2006; Karlsson et al., 2020). In particular, many *Sphingomonas* species are resistant to stress (e.g., desiccation, low nutrient levels) and produce exopolysaccharides (Glaeser and Kämpfer, 2014). These biopolymers can adhere to the surfaces, thus facilitating the attachment of different microorganisms and potentiating chemical and biological interaction in the stains (Ferrari et al., 2015). In addition, many *Sphingomonadaceae* are known for their ability to biodegrade a wide variety of organic substrates, such as chloroaromatic compounds, polycyclic aromatic hydrocarbons (common air pollutants in urban traffic areas) and several anthropogenic xenobiotics including biocides (Glaeser and Kämpfer, 2014; Mitra et al., 2020), which possibly favors the bio-colonization of the ETICS that contain organic compounds in the finishing coat. Other bacterial taxa found enriched in the ETICS stains' microbiota are also known to be highly proficient in metabolizing organic compounds, namely the *Acetobactereaceae* (Komagata et al., 2014), the *Kineosporiaceae* (Schellenberger et al., 2010; Jurado et al., 2011), the genus *Truepera* (*Trueperaceae*) (Albuquerque et al., 2005) and the genus *Rubellimicrobium* (*Rhodobactereaceae*) (Denner et al., 2006). At least some of these heterotrophic bacterial colonizers may potentially contribute to diverse forms of biodeterioration of the ETICS surface, namely (i) by producing pigments that contribute to colored aesthetic alterations (e.g., *Rubellimicrobium* spp., *Truepera* spp., *Kineosporeaceae*) (Albuquerque et al., 2005; Denner et al., 2006; Jurado et al., 2011); (ii) by promoting mechanical alterations associated with growth within already established fissures (Ferrari et al., 2015); (iii) by biodegrading components of the building materials and inducing chemical alterations due to formation of organic acid metabolites which can make materials more fragile (e.g., *Acetobactereaceae* acetic acid bacteria, *Kineosporeaceae* species, *Truepera* sp.) (Albuquerque et al., 2005; Schellenberger et al., 2010; Jurado et al., 2011; Komagata et al., 2014); and (iv) by metabolizing biocidal compounds, thus lessening their effectiveness (Hofbauer et al., 2011), which can contribute to enhance the intensity of biodeterioration phenomena.

Regarding the fungi community contribution to ETICS facade biodeterioration, the identified molds can form powdery-colored stains due to their pigmentation, with the potential to alter the facade's aesthetic appearance (Gaylarde and Gaylarde, 2005; Ferrari et al., 2015). Additional contributions of the filamentous fungi, either lichenized or free-living, for biodeterioration, may be due to hyphae penetration in fissures and in-depth growth into the substrate causing micro-tensions (Ferrari et al., 2015). Molds may also contribute to materials biodegradation, discoloration, cracking, general loss of strength, and enlargement of crevices, associated with potential structural anomalies, due to hydrolytic and oxidative enzymes excretion and production of organic acid metabolites (e.g., oxalic, acetic, and citric acids) (Gaylarde et al., 2011; Ferrari et al., 2015; Wu et al., 2022). Many of the mold genera identified in the ETICS stains have been detected as common colonizers and biodeteriogens of building surfaces, for instance (i) in cementitious building materials (e.g., *Cladosporium, Alternaria, Penicillium*) (Ferrari et al., 2015; Campana et al., 2020); (ii) in (sand) stone surfaces (e.g., *Cladosporium, Epicoccum, Penicillium, Knufia*) (Rosado et al., 2020; Wu et al., 2022); and (iii) in external painted surfaces (e.g., *Cladosporium, Epicoccum, Aureobasidium*) (Gaylarde and Gaylarde, 2005; Gaylarde et al., 2011; Ferrari et al., 2015). In general, these molds are ubiquitous in soil, rocks, decaying vegetation, trees, pollen, and dust (Honegger, 2009; van Nieuwenhuijzen et al., 2016; Chen et al., 2017; Abdollahzadeh et al., 2020; Liu et al., 2022) and their spores and mycelium fragments can undergo airborne dispersal, either outdoor or indoor (Karlsson et al., 2020; Savković et al., 2021). The high prevalence of the class *Lecanoromycetes* (orders *Lecanorales*, *Teloschistales*, and *Caliciales*) in the fungal microbiota of almost all the ETICS stains is noteworthy since these taxa have a widespread distribution and contain around 78% of the known lichen-forming fungi (Honegger, 2009; Lücking and Nelsen, 2018). In turn, green microalgae chlorophytes, also detected within the stains' microbiota, are photobiont partners in many lichenized ascomycete fungi (Honegger, 2009).

In summary, a microbiological succession may occur in the stains, with the potential to cause biodeterioration of ETICS facades. Primary biomass producers (e.g., cyanobacteria, microalgae) and/or producers of adhesive materials like extracellular polymers (e.g., microalgae, sphingomonads, etc.) can be pioneer organisms essential for the succession of heterotrophic microbiota in facade biofilms (Honegger, 2009; Komar et al., 2022; Wu et al., 2022). Those microbial materials can contribute to increasing not only the adhesion of cells and spores from a diversity of the identified bacteria and fungi but also the accumulation of dirt, plant debris, and/or pollutants. In turn, the latter may possibly contribute to accelerating and consolidating the formation of biofilms within the bio-colonized stains (Gaylarde and Gaylarde, 2005; Gaylarde et al., 2011; Ferrari et al., 2015; Wu et al., 2022). Given that many of the identified microorganisms can potentially induce chemical, mechanical, and aesthetic alterations on the ETICS surfaces and diverse microorganisms were found in the same bio-colonized stain, it is rather difficult to attribute biodeterioration patterns to specific microbial colonizers and further studies are needed. Next, omics analysis at transcriptome and metabolome levels, further confirmation with physiological studies, and correlation analysis regarding relevant *in situ* environmental parameters (Chen and Gu, 2022; Komar et al., 2022), may generate meaningful data to better understand the roles played by the active populations on discoloration, biodeterioration and biodegradation processes in the ETICS facades.

5. Conclusion

Our study reveals the unique microbial communities of color/aesthetic biodeterioration anomalies in ETICS facades, unlike those of other surfaces, and the associated environmental dynamics. Furthermore, the identification of diverse microbial colonizers with the potential for materials biodeterioration evidences the need to include additional microorganisms in the list of bio-susceptibility testing organisms in ETICS.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

ITS amplicon sequences of fungi isolates (GenBank) and raw nucleotide sequences of the microbiomes (SRA/BioProject accession ID PRJNA912626) will be released and made available upon paper publication

Acknowledgments

This work was supported by national funds from FCT - Fundação para a Ciência e a Tecnologia, I.P., Portugal, in the scope of the research project WGB_Shield (contract PTDC/ECI-EGC/30681/2017), the project UIDB/04625/2020 of the research unit Civil Engineering Research and Innovation for Sustainability - CERIS, the projects UIDB/04565/2020 and UIDP/04565/2020 of the research unit Institute for Bioengineering and Biosciences – iBB, the project LA/P/0140/2020 of the Associate Laboratory Institute for Health and Bioeconomy - i4HB, and the Ph.D. scholarship DFA/BD/5180/2020 (J. L. Parracha). The authors acknowledge Ricardo Leite (IGC, Oeiras, PT) for help in the analysis of plastid/chloroplast sequences, Ing. Luís Brás (Gebalis S.A., Lisboa, PT) and Prof. João Gomes Ferreira (IST-UL, Lisboa, PT) for the opportunity to analyze the case studies, and Ricardo Deus and IPMA (Portuguese Institute for Sea and Atmosphere) for meteorological data.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ibiod.2023.105658.

References

Abarenkov, K., Zirk, A., Piirmann, T., Pöhönen, R., Ivanov, F., Nilsson, R.H., Kõljalg, U., 2021. UNITE QIIME Release for Fungi. UNITE Community. https://doi.org/10.15156/BIO/1264708. Version 10.05.2021.

Abdollahzadeh, J., Groenewald, J.Z., Coetzee, M.P.A., Wingfield, M.J., Crous, P.W., 2020. Evolution of lifestyles in capnodiales. Stud. Mycol. 95, 381–414. https://doi.org/10.1016/j.simyco.2020.02.004.

Albuquerque, L., Simões, C., Nobre, M.F., Pino, N.M., Battista, J.R., Silva, M.T., Rainey, F.A., da Costa, M.S., 2005. *Truepera radiovictrix* gen. nov., sp. nov., a new radiation resistant species and the proposal of *Trueperaceae* fam. nov. FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Lett. 247, 161–169. https://doi.org/10.1016/j.femsle.2005.05.002.

Amaro, B., Saraiva, D., De Brito, J., Flores-Colen, I., 2013. Inspection and diagnosis system of ETICS on walls. Construct. Build. Mater. 47, 1257–1267. https://doi.org/10.1016/j.conbuildmat.2013.06.024.

Amaro, B., Saraiva, D., De Brito, J., Flores-Colen, I., 2014. Statistical survey of the pathology, diagnosis and rehabilitation of ETICS in walls. J. Civ. Eng. Manag. 20, 511–526. https://doi.org/10.3846/13923730.2013.801923.

Antošová, N., 2013. Impact of biocorrosion on the durability of ETICS and empirical findings about the periodicity of maintenance. Slovak Journal of Civil Engineering 21, 21–28. https://doi.org/10.2478/sjce-2013-0009.

Apprill, A., McNally, S., Parsons, R., Weber, L., 2015. Minor revision to V4 region SSU rRNA 806R gene primer greatly increases detection of SAR11 bacterioplankton. Aquat. Microb. Ecol. 75, 129–137. https://doi.org/10.3354/ame01753.

Barberousse, H., Ruot, B., Yéprémian, C., Boulon, G., 2007. An assessment of façade coatings against colonisation by aerial algae and cyanobacteria. Build. Environ. 42, 2555–2561. https://doi.org/10.1016/j.buildenv.2006.07.031.

Barreira, E., de Freitas, V.P., 2013. Experimental study of the hygrothermal behaviour of external thermal insulation composite systems (ETICS). Build. Environ. 63, 31–39. https://doi.org/10.1016/j.buildenv.2013.02.001.

Bokulich, N.A., Kaehler, B.D., Rideout, J.R., Dillon, M.R., Bolyen, E., Knight, R., Huttley, G.A., Caporaso, J.G., 2018. Optimizing taxonomic classification of marker-gene amplicon sequences with QIIME 2's q2-feature-classifier plugin. Microbiome 6, 90. https://doi.org/10.1186/s40168-018-0470-z.

Bolyen, E., Rideout, J.R., Dillon, M.R., Bokulich, N.A., Abnet, C.C., Al-Ghalith, G.A., Alexander, H., Alm, E.J., Arumugan, M., Asnicar, F., et al., 2019. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat. Biotechnol. 37, 852–857. https://doi.org/10.1038/s41587-019-0209-9.

Burkhardt, M., Zuleeg, S., Vonbank, R., Schmid, P., Hean, S., Lamani, X., Bester, K., Boller, M., 2011. Leaching of additives from construction materials to urban stormwater runoff. Water Sci. Technol. 63, 1974–1982. https://doi.org/10.2166/wst.2011.128.

Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J.A., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nat. Methods 13, 581–583. https://doi.org/10.1038/nmeth.3869.

Campana, R., Sabatini, L., Frangipani, E., 2020. Moulds on cementitious building materials – problems, prevention and future perspectives. Appl. Microbiol. Biotechnol. 104, 509–514. https://doi.org/10.1007/s00253-019-10185-7.

Chen, J., Gu, J.-D., 2022. The environmental factors used in correlation analysis with microbial community of environmental and cultural heritage samples. Int. Biodeterior. Biodegrad. 173, 105460 https://doi.org/10.1016/j.ibiod.2022.105460.

Chen, Q., Hou, L.W., Duan, W.J., Crous, P.W., Cai, L., 2017. *Didymellaceae* revisited. Stud. Mycol. 87, 105–159. https://doi.org/10.1016/j.simyco.2017.06.002.

Coombs, K., Vesper, S., Green, B.J., Yermakov, M., Reponen, T., 2017. Fungal microbiomes associated with green and non-green building materials. Int. Biodeterior. Biodegrad. 125, 251–257. https://doi.org/10.1016/j.ibiod.2017.07.018.

Coutinho, M.L., Miller, A.Z., Gutierrez-Patricio, S., Hernandez-Marine, M., Gomez-Bolea, A., Rogerio-Candelera, M.A., Philips, A.J.L., Jurado, V., Saiz-Jimenez, C., Macedo, M.F., 2013. Microbial communities on deteriorated artistic tiles from pena national palace (sintra, Portugal). Int. Biodeterior. Biodegrad. 84, 322–332. https://doi.org/10.1016/j.ibiod.2012.05.028.

Coutinho, M.L., Miller, A.Z., Macedo, M.F., 2015. Biological colonization and biodeterioration of architectural ceramic materials: an overview. J. Cult. Herit. 16, 759–777. https://doi.org/10.1016/j.culher.2015.01.006.

Crispim, C.A., Gaylarde, P.M., Gaylarde, C.C., Neilan, B.A., 2006. Deteriogenic cyanobacteria on historical buildings in Brazil detected by culture and molecular techniques. Int. Biodeterior. Biodegrad. 57, 239–243. https://doi.org/10.1016/j.ibiod.2006.03.001.

Denner, E.B.M., Kolari, M., Hoornstra, D., Tsitko, I., Kämpfer, P., Busse, H.-J., Salkinoja-Salonen, M., 2006. *Rubellimicrobium thermophilum* gen. nov., sp. nov., a red-pigmented, moderately thermophilic bacterium isolated from coloured slime deposits in paper machines. Int. J. Syst. Evol. Microbiol. 56, 1355–1362. https://doi.org/10.1099/ijs.0.63751-0.

Digel, I., Akimbekov, N.S., Kistaubayeva, A., Zhubanova, A.A., 2018. Microbial sampling from dry surfaces: current challenges and solutions. In: Artmann, G., Artmann, A., Zhubanova, A., Digel, I. (Eds.), Biological, Physical and Technical Basics of Cell Engineering. Springer, Singapore. https://doi.org/10.1007/978-981-10-7904-7_19.

D'Orazio, M., Cursio, G., Graziani, L., Aquilanti, L., Osimani, A., Clementi, F., Yéprémian, C., Lariccia, V., Amoroso, S., 2014. Effects of water absorption and surface roughness on the bioreceptivity of ETICS compared to clay bricks. Build. Environ. 77, 20–28. https://doi.org/10.1016/j.buildenv.2014.03.018.

EAD, 2020. Guideline for European Technical Approval of External Thermal Insulation Composite Systems with Rendering (EAD 040083-00-0404), Superseding ETAG 004. Published by EOTA (European Organisation for Technical Approval), Brussels, Belgium. Available online at: https://www.eota.eu/eads. (Accessed 10 July 2023).

EAE-European Association for ETICS, 2011. European guideline for the application of ETICS, EAE, Baden, Germany. : https://www.ea-etics.com/wp-content/uploads/2021/08/EU_Application-Guideline_2011-03_EN.pdf. (Accessed 15 July 2022) https://www.ea-etics.com/publications-events/brochures/. Available online at.

EAE-European Association for ETICS, 2021. The European ETICS market 2021, EAE, Baden, GE. Available online at: https://www.ea-etics.com/publications-events/etics-forum-2021/. (Accessed 18 July 2022).

ETAG, 2000. ETAG 004 - Guideline for European Technical Approval of External Thermal Insulation Composite Systems with Rendering. Published by EOTA (European Organisation for Technical Approval), Brussels, Belgium. Available online at: https://www.eota.eu/etags-archive. (Accessed 10 July 2023).

EU, 2014. Regulation (EU) N. 528/2012 of the European Parliament and of the Council Concerning the Making Available on the Market and Use of Biocidal Products. 22 May 2012, amended and corrected in 25 May 2014.

Ferrari, C., Santunione, G., Libbra, A., Muscio, A., Sgarbi, E., Siligardi, C., Barozzi, G.S., 2015. Review of the influence of biological deterioration on the surface properties of building materials: organism, materials and methods. Int. J. Des. Nat. Ecodyn. 10, 21–39. https://doi.org/10.2495/DNE-V10-N1-21-39.

Gaylarde, C.C., Gaylarde, P.M., 2005. A comparative study of the major microbial biomass of biofilms on exteriors of buildings in Europe and Latin America. Int. Biodeterior. Biodegrad. 55, 131–139. https://doi.org/10.1016/j.ibiod.2004.10.001.

Gaylarde, C.C., Morton, L.H.G., Loh, K., Shirakawa, M.A., 2011. Biodeterioration of external architectural paint films - a review. Int. Biodeterior. Biodegrad. 65, 1189–1198. https://doi.org/10.1016/j.ibiod.2011.09.005.

Glaeser, S.P., Kämpfer, P., 2014. The family *Sphingomonadaceae*. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-30197-1_302.

Hofbauer, W., Krueger, N., Mayer, F., Breuer, K., 2011. Biocide tolerance in microorganisms with respect to the durability of building coatings. In: Proceedings of XII DBMC- International Conference on Durability of Building Materials and Components. Porto, Portugal, pp. 93–99, 12-15 April.

Honegger, R., 2009. Lichen-forming fungi and their photobionts. In: Deising, H. (Ed.), Plant Relationships, the Mycota V. Springer-Verlag, Berlin, Heidelberg, pp. 308–333. https://doi.org/10.1007/978-3-540-87407-2_16.

Johansson, S., 2011. Microbial Growth on Buildings Facades with Thin Rendering on Thermal Insulation. *Ph.D. Thesis, Report TVBM-1029*, Division of Building Materials. University of Lund, Sweden.

Jurado, V., Laiz, L., Ortiz-Martinez, A., Groth, I., Saiz-Gimenez, C., 2011. *Pseudokineococcus lusitanus* gen. nov., sp. nov., and reclassification of *Kineococcus marinus* Lee 2006 as *Pseudokineococcus* marinus comb. Nov. International Journal of Systematic and Evolutionary Microbioliology 61, 2515–2519. https://doi.org/10.1099/ijs.0.026195-0.

Karlsson, E., Johansson, A.-M., Ahlinder, J., Lundkvist, M.J., Singh, N.J., Brodin, T., Forsman, M., Stenberg, P., 2020. Airborne microbial diversity and seasonality in northern and southern sweeden. Peer Journal 8, e8424. https://doi.org/10.7717/peerj.8424.

Komagata, K., Iino, T., Yamada, Y., 2014. The family *Acetobacteraceae*. In: Rosenberg, E., DeLong, E.F., Lory, S., Stackebrandt, E., Thompson, F. (Eds.), The Prokaryotes. Springer, Berlin, Heidelberg. https://doi.org/10.1007/978-3-642-30197-1_396.

Komar, M., Nowicka-Krawczyk, P., Ruman, T., Nizioł, J., Konca, P., Gutarowska, B., 2022. Metabolomic analysis of photosynthetic biofilms on building façades in temperate climate zones. Int. Biodeterior. Biodegrad. 169, 105374 https://doi.org/10.1016/j.ibiod.2022.105374.

Künzel, H., Künzel, H.M., Sedlbauer, K., 2006. Long-term performance of external thermal insulation systems (ETICS). Architectura 5, 11–24.

Liu, B., Fu, R., Wu, B., Liu, X., Xiang, M., 2022. Rock inhabiting fungi: terminology, diversity, evolution, and adaptation mechanisms. Mycology 13, 1–31. https://doi.org/10.1080/21501203.2021.2002452.

Lücking, R., Nelsen, M.P., 2018. Chapter 23 - ediacarans, Protolichens, and Lichen-Derived *Penicillium*: a critical reassessment of the evolution of lichenization in fungi. In: Krings, M., Cúneo, N.R., Harper, C.J., Rothwell, G. (Eds.), Transformative Paleobotany. Elsevier, pp. 551–590. https://doi.org/10.1016/b978-0-12-813012-4.00023-1.

McDonald, D., Price, M.N., Goodrich, J., Nawrocki, E.P., DeSantis, T.Z., Probst, A., Andersen, G.L., Knight, R., Hugenholtz, P., 2012. An improved Greengenes taxonomy with explicit ranks for ecological and evolutionary analyses of bacteria and archaea. ISME J. 6, 610–618. https://doi.org/10.1038/ismej.2011.139.

Mislivec, P.B., Bruce, V.R., 1976. Comparison of antibiotic-amended potato dextrose agar and acidified potato dextrose agar as growth substrates for fungi. J. Assoc. Off. Anal. Chem. 59, 720–721. https://doi.org/10.1093/jaoac/59.3.720.

Mitra, M., Nguyen, K.M.-A.-K., Box, T.W., Gilpin, J.S., Hamby, S.R., Berry, T.L., Duckett, E.H., 2020. Isolation and characterization of a novel Sphingobium yanoikuyae strain variant that uses biohazardous saturated hydrocarbons and aromatic compounds as sole carbon sources. F1000Research 9, 767. https://doi.org/10.12688/f1000research.25284.1.

Nienow, J.A., 2009. Extremophiles: dry environments (including crytoendoliths). In: Schaechter, M. (Ed.), Encyclopedia of Microbiology, third ed. Academic Press.

Nilsson, R.H., Larsson, K.-H., Taylor, A.F.S., Bengtsson-Palme, J., Jeppesen, T.S., Schigel, D., Kennedy, P., Picard, K., Glöckner, F.O., Tedersoo, L., Saar, I., Kõljalg, U., Abarenkov, K., 2019. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 47, D259–D264. https://doi.org/10.1093/nar/gky1022.

Parada, A.E., Needham, D.M., Fuhrman, J.A., 2016. Every base matters: assessing small subunit rRNA primers for marine microbiomes with mock communities, time series and global field samples. Environ. Microbiol. 18, 1403–1414. https://doi.org/10.1111/1462-2920.13023.

Parracha, J.L., Borsoi, G., Flores-Colen, I., Veiga, R., Nunes, L., Dionísio, A., Gomes, M.G., Faria, P., 2021a. Performance parameters of ETICS: correlating water resistance, bio-susceptibility and surface properties. Construct. Build. Mater. 272, 121956 https://doi.org/10.1016/j.conbuildmat.2020.121956.

Parracha, J.L., Borsoi, G., Veiga, R., Flores-Colen, I., Nunes, L., Garcia, A.R., Ilharco, L., Dionísio, A., Faria, P., 2021b. Effects of hygrothermal, UV and SO_2 accelerated ageing on the durability of ETICS in urban environments. Build. Environ. 204, 108151 https://doi.org/10.1016/j.buildenv.2021.108151.

Parracha, J.L., Borsoi, G., Flores-Colen, I., Veiga, R., Nunes, L., 2022. Impact of natural and artificial ageing on the properties of multilayer external wall thermal insulation systems. Construct. Build. Mater. 317, 125834 https://doi.org/10.1016/j.conbuildmat.2021.125834.

Parracha, J.L., Veiga, R., Flores-Colen, I., Nunes, L., 2023. Toward the sustainable and efficient use of External Thermal Insulation Composite Systems (ETICS): a comprehensive review of anomalies, performance parameters, requirements and durability. Buildings 13, 1664. https://doi.org/10.3390/buildings13071664.

Reiß, F., Kiefe, N., Noll, M., Kalkhof, S., 2021. Application, release, ecotoxicological assessment of biocide in building materials and its soil microbial response. Ecotoxicol. Environ. Saf. 224, 112707 https://doi.org/10.1016/j.ecoenv.2021.112707.

Rosado, T., Mirão, J., Candeias, A., Caldeira, A.T., 2014. Microbial communities analysis assessed by pyrosequencing - a new approach applied to conservation state studies of mural paintings. Anal. Bioanal. Chem. 406, 888–895. https://doi.org/10.1007/s00216-013-7516-7.

Rosado, T., Dias, L., Lança, M., Nogueira, C., Santos, R., Martins, M.R., Candeias, A., Mirão, J., Caldeira, A.T., 2020. Assessment of microbiota present on a Portuguese historical stone convent using high-throughput sequencing approaches. Microbiol. 9, e1030 https://doi.org/10.1002/mbo3.1030.

Savković, Ž., Stupar, M., Unković, N., Ivanović, Ž., Blagojević, J., Popović, S., Vukojević, J., Grbić, M.L., 2021. Diversity and seasonal dynamics of culturable airborne fungi in a cultural heritage conservation facility. Int. Biodeterior. Biodegrad. 157, 105163 https://doi.org/10.1016/j.ibiod.2020.105163.

Schellenberger, S., Kolb, S., Drake, H.L., 2010. Metabolic responses of novel cellulolytic and saccharolytic agricultural soil bacteria to oxygen. Environ. Microbiol. 12, 845–861. https://doi.org/10.1111/j.1462-2920.2009.02128.x.

Shirakawa, M.A., Loh, K., John, V.M., Silva, M.E.S., Gaylarde, C.C., 2011. Biodeterioration of painted mortar surfaces in tropical urban and coastal situations: comparison of four paint formulations. Int. Biodeterior. Biodegrad. 65, 669–674. https://doi.org/10.1016/j.ibiod.2011.03.004.

van Nieuwenhuijzen, E.J., Houbraken, J.A.M.P., Meijer, M., Adan, O.C.G., Samson, R.A., 2016. Aureobasidium melanogenum: a native of dark biofinishes on oil treated wood. Antonie Leeuwenhoek 109, 661–683. https://doi.org/10.1007/s10482-016-0668-7.

Walters, W., Hyde, E.R., Berg-Lyons, D., Ackermann, G., Humphrey, G., Parada, A., Gilbert, J.A., Jansson, J.K., Caporaso, J.G., Fuhrman, J.A., Apprill, A., Knight, R., 2015. Improved bacterial 16S rRNA gene (V4 and V4-5) and fungal internal transcribed spacer marker gene primers for microbial community surveys. mSystems 1, e00009. https://doi.org/10.1128/mSystems.00009-15, 15.

Wang, Y., Huang, W., Han, Y., Huang, X., Wang, C., Ma, K., Kong, M., Jiang, N., Pan, J., 2022. Microbial diversity of archaeological ruins of Liangzhu City and its correlation with environmental factors. Int. Biodeterior. Biodegrad. 175, 105501 https://doi.org/10.1016/j.ibiod.2022.105501.

Wu, F., Zhang, Y., Gu, J.-D., He, D., Zhang, G., Liu, X., Guo, Q., Cui, H., Zhao, J., Feng, H., 2022. Community assembly, potential functions and interactions between fungi and microalgae associated with biodeterioration of sandstone at the Beishiku Temple in Northwest China. Sci. Total Environ. 835, 155372 https://doi.org/10.1016/j.scitotenv.2022.155372.

Ximenes, S., De Brito, J., Gaspar, P.L., Silva, A., 2015. Modelling the degradation and service life of ETICS in external walls. Mater. Struct. 48, 2235–2249. https://doi.org/10.1617/s11527-014-0305-8.